

BIOGRAPHICAL SKETCH

NAME: Li, Senlin

eRA COMMONS USER NAME (credential, e.g., agency login): LISENLIN

POSITION TITLE: Professor of Medicine

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Shanxi Medical College, Taiyuan, PR China		1982	
Haerbin Medical University, Haerbin City, PR China	M. Med.	1985	Medicine
Peking Union Medical College, Peking, PR China		1989	Biochemistry and Molecular Biology
University of Geneva School of Medicine, Geneva, Switzerland	M.D.	1991	Medicine
Shanxi Medical College, Taiyuan, PR China		1982	

A. Personal Statement

The goal of the proposed research is to continue our translational study toward developing novel therapy to fight aging and age-related diseases. For the past decade, my research has been focused on exploration of potential hematopoietic stem cell (HSC) transplantation-based gene therapy for two prevalent age-related diseases – Parkinson's disease (PD) and atherosclerosis. Macrophages – an HSC progeny cell type – are used to deliver glial cell line-derived neurotrophic factor (GDNF) to the degenerating substantia nigra dopamine neurons in order to develop a neuro-protective/disease-modifying treatment for PD. This will be achieved by lentiviral HSC gene therapy. Macrophages are also applied in this format to ameliorate atherosclerosis by over-expression of atheroprotective genes such as apoE and LXRA and/or knockdown of atherogenic genes. In these investigations, manipulation of HSCs and their effective transplantation and repopulation of the hematopoietic system are key requirements for success. In order to achieve engraftment of transplanted HSCs in the host, the existing endogenous HSCs first have to be removed, just as in solid tissue/organ transplantation. Irradiation and/or chemotherapy are conventionally used to remove/destroy the to-be-replaced HSCs. The harsh transplant conditioning procedures, certainly negatively affecting the health and life span of the recipients, has strictly limited HSC transplantation to life-threatening diseases and preclinical research. To find a gentle conditioning method is the dream of hematologists, as well as stem cell/gene therapy researchers. After many years of work, we recently developed a transformative conditioning regimen – gentle and completely free of irradiation and use of chemotherapeutic agents. The final transplantation rates consistently reach ~90%. We are very excited about this invention and eager to apply it in anti-aging research and therapy. We hypothesize that rejuvenation of HSCs will lead to prolonged healthspan and lifespan. We have assembled a very talented research team committed to the successful execution of this project at the Barshop Institute. Stem cell rejuvenation will be developed as a novel intervention to enhance healthy aging and increase lifespan. The approach may also apply to Alzheimer's disease (AD), potentially leading to a disease-modifying therapy for AD. Specifically, neurotrophic factors such as GDNF for PD or BDNF for AD will be delivered to all or most of the degenerating neurons by infiltrating macrophages that become microglia-like cells

locally. I was trained initially as a physician. Later on I received excellent training in molecular biology, including gene cloning, vector construction, transcription control, gene expression, promoter activity, synthetic promoters, and so on. Since I became an independent PI, I have been intensively focused on stem cells, especially the harnessing of hematopoietic stem cells to deliver neurotrophic factors to neurodegenerative sites. Stem cell rejuvenation comprises a novel intervention to enhance healthy aging and increase lifespan.

B. Positions and Honors

Positions and Employment

1986-1989	Lecturer, Department of Child and Adolescent Health, Shanxi Medical College, Taiyuan, PR China
1989-1991	Investigator in Clinical Endocrinology, University of Geneva, Switzerland
1991-1992	Postdoctoral Fellow, Division of Clinical Biochemistry, University of Geneva, Switzerland
1992-1995	Postdoctoral Fellow, Fondation pour Recherches Medicales, University of Geneva, Switzerland
1995-1999	Senior Postdoctoral Fellow and Research Scientist, Department of Medicine, University of Texas Health Science Center, San Antonio, TX
2000-2006	Assistant Professor of Medicine & Barshop Center for Longevity and Aging Studies, UTHSCSA
2006-2014	Associate Professor of Medicine & Barshop Center for Longevity and Aging Studies, UTHSCSA
2014-Present	Professor of Medicine & Barshop Center for Longevity and Aging Studies, UTHSCSA
2000-present	Research Health Scientist (Health Science Specialist), South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, TX

Professional Memberships

1998-2014	Member, American Association for the Advancement of Science
2004-present	Member, American society of Gene therapy

Honors

1989-1991	World Health Organization Scholarship, University of Geneva, Switzerland
2002	Winner, Best Junior Faculty Poster, Department of Medicine 5 th Annual Research Day, University of Texas Health Science Center, San Antonio, TX

Patents

Li S, and Clark RA. United States Patent No. 7,709,625 B2 issued May 4, 2010 entitled: Methods and compositions for bone marrow stem cell-derived macrophage delivery of genes for gene therapy.

UTHSCSA/STTM Reference No.: 2004.006.HSCS

Li S, Clark RA, and Chen C. United States Provisional Patent Application (HSCSA0033USP2) on May 8, 2014 entitled: Methods and compositions for non-cytotoxic stem cell transplantation.

C. Contributions to Science

1. Promoter activity and gene regulation of NADPH oxidase – PU.1 as a major player: The microbicidal NADPH oxidase system of phagocytic leukocytes, comprising gp91*phox*, p47*phox*, p67*phox*, p40*phox*, and other co-factors, is required for normal host defenses against a wide variety of bacterial and fungal pathogens, as is vividly illustrated by the phenotype of severe recurrent infections in chronic granulomatous disease (CGD), a group of disorders caused by

mutations in the genes for one or another of these factors. In addition to mutations in the coding regions, transcriptional dysregulation was also shown to cause the disease. Therefore, I extensively studied how expression of these factors is transcriptionally controlled when I was a post-doc fellow. Using various molecular biological techniques such as DNase I footprint, electrophoretic mobility shift assay (EMSA), reporter assay, and chromatin immunoprecipitation (ChIP), I found that PU.1, an ETS-domain transcription factor, is required for transcriptional control of these NADPH oxidase subunits. However, this is achieved by different means. For example, PU.1 binds to the very proximal region of *p47phox* promoter (from -40 to -45) and is essential for the promoter activity. We further showed that positions -46 and -47 are also critical to the full promoter activity. At the *p40phox* locus, there are multiple PU.1 binding sites that are required for gene transcription in myeloid cells. Granulocytic differentiation is associated with the coordinated up-regulation of PU.1 and *p40phox* expression. In the case of *p67phox*, promoter activity requires cooperation between PU.1 and non-myeloid transcription factors, with AP-1 being the most critical for function.

- a. **Li S**, Valente AJ, Zhao S-J, and Clark RA. PU.1 is essential for *p47phox* promoter activity in myeloid cells. *J Biol Chem* 272:17802-17809, 1997.
- b. **Li S**, Schlegel W, Valente AJ, and Clark RA. Critical flanking sequences of PU.1 binding sites in myeloid-specific promoters. *J Biol Chem* 274:32453-32460, 1999.
- c. **Li S**, Valente AJ, Wang L, Gamez MJ, and Clark RA. Transcriptional regulation of the *p67phox* gene: Role of AP-1 in concert with myeloid-specific transcription factors. *J Biol Chem* 276:39368-39378, 2001.
- d. **Li S**, Valente AJ, and Clark RA. Multiple PU.1 binding is required for *p40phox* gene transcription in myeloid cells. *Blood* 99:4578-4587, 2002.

2. Synthetic myeloid/macrophage promoters: Myeloid-specific *cis* elements for PU.1, C/EBP α , and AML-1 and myeloid-associated *cis* elements for Sp1 and AP-1 identified by us and others were randomly ligated and inserted into a luciferase reporter construct upstream of the *p47phox* basal promoter to generate a library of synthetic promoters. The library was initially screened for promoter activity in a human myeloid cell line. Later, the lead sequences were further tested for macrophage or neutrophilic specificity. Several macrophage promoters were characterized (patented); one of them has been employed extensively by us and other investigators. In my lab, the synthetic macrophage has been used in hematopoietic stem cell transplantation-based macrophage-mediated gene therapy as described below. In gene therapy, there is generally a pressing need for strong tissue- or cell-specific promoters.

- a. He W, Qiang M, Ma W, Valente AJ, Quinones M, Wang W, Reddick, RL, Qifu X, Ahuja SS, Clark RA, Freeman GL, and **Li S**. Development of a synthetic promoter for macrophage gene therapy. *Human Gene Therapy* 11: 949-959, 2006.
- b. A-Gonzalez N, Guillen JA, Gallardo G, Diaz M, de la Rosa JV, Hernandez IH, Casanova-Acebes M, Lopez F, Tabraue C, Beceiro S, Hong C, Lara PC, Andujar M, Arai S, Miyazaki T, **Li S**, Corbi AL, Tontonoz P, Hidalgo A, Castrillo A. The Nuclear Receptor LXR α controls the functional specialization of splenic macrophages. *Nature Immunology* 14: 831-839, 2013.

3. Hematopoietic stem cell transplantation-based macrophage-mediated neurotrophic factor brain delivery for Parkinson's disease – an application of the synthetic promoter: Neurotrophic factors are neuroprotective and neurorestorative, but their delivery to CNS is challenging due to blood-brain barrier and their poor diffusibility in the brain tissue. Macrophages derived from bone marrow home to degenerating sites of brain, thus making them an excellent option for delivery of neurotrophic factors. Using two mouse models of PD, we demonstrated that genetically engineered HSC-derived macrophages accumulated selectively in diseased sites and macrophage-mediated neurotrophic factor (GDNF, glial cell line-derived neurotrophic factor, or NTN, neurturin) delivery dramatically ameliorated degeneration of tyrosine hydroxylase (TH)-positive dopaminergic neurons of the substantia nigra and TH+ terminals in the striatum, stimulated axon regeneration, and reversed hypoactivity, without apparent adverse effects. Recently, we tested this therapeutic approach in a chronic and progressive PD model – the MitoPark mouse. Again, transgene-

expressing macrophages infiltrated the CNS and delivered GDNF to the midbrain of MitoPark mice, but this did not occur in the normal littermates. Macrophage-mediated GDNF delivery dramatically reduced the loss of DA neurons in the substantia nigra and TH-positive terminals in the striatum, while markedly improving their motor as well as non-motor dysfunction. This work was selected as the first place winner for 2011 TINT (Technology Innovation in Novel Translation) Program at UTHSCSA. As I stated in a 2013 invited commentary in *Science News* magazine regarding a pair of landmark publications in *Science* of lentiviral hematopoietic stem cell gene therapy clinical trials, “scientists may start doing gene therapy for common conditions such as Parkinson’s disease.” We are moving toward testing in primate models, and will progress eventually to human clinical trials.

- a. Biju KC, Zhou Q, Li G, Imam SZ, Roberts RL, Morgan WW, Clark RA, and **Li S**. Macrophage-mediated GDNF delivery protects against dopaminergic neurodegeneration: A therapeutic strategy for Parkinson’s disease. *Molecular Therapy* 18:1536-1544, 2010.
- b. Imam SZ, Zhou Q, Yamamoto A, Valente AJ, Ali SF, Bains MC, Roberts JL, Kahle PJ, Clark RA, **Li S**. Novel Regulation of Parkin Function Through c-Abl-Mediated Tyrosine Phosphorylation: Implications for Parkinson’s Disease. *Journal of Neuroscience* 31: 157–163, 2011.
- c. Biju KC, Santacruz RA, Chen C, Zhou Q, Yao J, Rohrabough SL, Clark RA, Roberts JL, Phillips KA, **Li S**. Bone marrow-derived microglia based neurturin delivery protects against dopaminergic neurodegeneration in a mouse model of Parkinson’s disease. *Neurosci Lett* 535:24-29, 2013.
- d. Branch SY, Chen C, Sharma R, Lechleiter JD, **Li S**, Beckstead MJ. Dopaminergic Neurons Exhibit an Age-Dependent Decline in Electrophysiological Parameters in the MitoPark Mouse Model of Parkinson’s Disease. *Journal of Neuroscience* 36:4026-37, 2016.
- e. Chen C, Liu J, Li X, Xue X, Biju KC, Liang SD, Qian Y, Ballard C, O’Connor JC, Masliah E, Clark RA, and **Li S**. Macrophage-mediated GDNF gene therapy protects against dopaminergic neurodegeneration and improves motor and non-motor dysfunction in MitoPark mouse model of Parkinson’s disease. Manuscript submission to *PNAS*.

4. Hematopoietic stem cell transplantation-based macrophage gene therapy of atherosclerosis: Atherosclerosis is a disorder of lipid metabolism, as well as chronic inflammation. Macrophages, participating in both of these processes, are intimately involved in all phases of atherosclerosis, from development of the fatty streak to processes that ultimately contribute to plaque rupture and myocardial infarction. Macrophage expression of certain genes may protect against atherosclerosis and low or absent expression of these genes leads to atherogenesis. Such genes include apoE, apoA1, ABCA1, HSL, LXR, and PPAPy, among others. On the other hand, some genes expressed in macrophages promote atherogenesis, such as PPARd, CCR2, MCP-1, CCR5, MCSF, COX-2, 12/15-LO, and macrophage fatty-acid-binding protein aP2. Transgenic over-expression of beneficial genes or knock-down/out of detrimental genes in macrophages is expected to mitigate atherosclerosis. Hematopoietic stem cell transplantation-based macrophage-mediated gene therapy may provide lifelong anti-atherogenic macrophages and thus keep atherosclerotic plaques at low levels.

- a. Li G, Biju KC, Xu X, Zhou Q, Chen C, Valente AJ, He W, Reddick RL, Freeman GL, Ahuja SS, Clark RA and **Li S**. Macrophage LXR α gene therapy ameliorates atherosclerosis as well as hypertriglyceridemia in LDLR $^{-/-}$ mice. *Gene Therapy* 18: 835–841, 2011.
- b. Li G, Chen C, Liang SD, Ballard C, Biju KC, Reddick RL, Clark RA and **Li S**. Hematopoietic knockdown of PPAR α reduces atherosclerosis in LDLR $^{-/-}$ mice. *Gene Therapy* 23:78-85, 2016.

5. Novel hematopoietic stem cell transplantation: In conventional HSCT, the necessary transplant pre-conditioning is achieved by cytotoxic/destructive chemotherapeutics (or irradiation) to clear/empty the HSC niches in the bone marrow. The contemporary HSCT procedures are thus feasible only for malignancies and other rapidly deteriorative conditions. Alternative gentle conditioning regimens are being actively sought. All current experimental conditioning regimens,

including antibody-based, type I interferon-mediated¹⁰, and G-CSF-modulated pre-transplant conditioning, still require cyto-destructive / reductive irradiation or chemotherapy, although at reduced doses. An exception is the c-kit antibody method, which can lead to chimerism levels in mice of up to 90% after subsequent transplantation with labeled HSCs and is irradiation- and chemotherapy-independent. Unfortunately, this method works only in immune-deficient mice, but not in immunocompetent individuals. In sharp contrast, we have been developing a novel conditioning regimen that is effective in fully immunocompetent animals and, moreover, is non-cytotoxic and thus free of the usual side effects of cyto-reductive approaches as described in the current proposal.

A List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/bibliography/48325609/>

D. Research Support (OTHER SUPPORT)

ACTIVE

Department of Veterans Affairs	Li (PI)	01/01/2016 – 12/31/2020
Merit Review	\$150,000/year	Effort: 5.0 calendar months

Macrophage-mediated gene delivery of neurotrophic factors for Parkinson's disease
 This project is to develop a lentiviral hematopoietic stem cell gene therapy to deliver GDNF (glial cell-derived neurotrophic factor) to degenerating neurons by macrophage infiltration to the diseased areas in various mouse PD models.
 Role: PI – Dr. Li' salary will be covered for 87.5% by additional VA money, so the amount listed is used to other lab people and research supplies.

OVERLAP: There is no budgetary overlap.

William and Ella Owens	Li (PI)	01/01/2016 – 12/31/2017
Medical Research Foundation	\$85,000/year	Effort: 2.4 calendar months

Development of a novel disease-modifying therapy for Parkinson's patients
 This proposal is to study the novel cell-based strategy of GDNF delivery for Parkinson's disease (PD) by emphasis on genotoxic effects on blood cells by longitudinal analysis of lentiviral vector insertion, clonal hematopoiesis, and vector genotoxicity.
 Role: PI

OVERLAP: There is little scientific and no budgetary overlap.

Office of Technology Commercialization	Li (PI)	01/01/2017 – 08/31/2017
Pilot Grant	\$25,000	Effort: 1.2 calendar months

Proof of concept study of a cure for chronic granulomatous disease
 Based on gene editing and a novel safe hematopoietic stem cell transplantation technology, this project is to provide proof of concept data in gp91^{-/-} mouse model of chronic granulomatous disease that a cure is achievable

CTSA/IIMS	Li (PI)	08/01/2015 – 05/30/2017
Pilot Grant	\$50,000	Effort: 1.2 calendar months

Disruption of CCR5 gene in blood stem cells followed by transplantation as curative therapy for HIV
 The major goals of this proposal are to develop a potential curative therapy for HIV/AIDS using a novel gentle hematopoietic stem cell transplantation procedure to populate hematopoietic blood cells of CCR5^{-/-} achieved with the latest CRISPR/Cas9 RNA-guided genome editing technology.

PENDING

1 R01 HL139768-01	Li (PI)	12/01/2017 – 11/30/2022
NIH/NHLBI	\$2,289,559.00	Effort: 4.6 calendar months

Validation of a non-cytotoxic clinically applicable hematopoietic stem cell transplant platform in large animals This proposal is to test the hypotheses that effective and stable engraftment of hematopoietic stem cells can be achieved by multiple cycles of apheresis and mobilization-enabled transplantation pig and monkey models.

Role: PI

OVERLAP: There is little scientific and no budgetary overlap.

COMPLETED

5R01DE015857-08

Werner (PI)

08/01/2008 – 07/31/2013

NIH/NIDCR

CSF-1 in Dental Biology

This proposal is to study the hypotheses: 1) cell-specific cis-acting elements in the -774 bp CSF-1 promoter direct gene expression in ameloblast lineage cells during tooth development, 2) CSF-1 isoforms differentially regulate enamel matrix and root formation and result in distinct phenotypes, and 3) lentiviral-mediated gene delivery of sCSF-1 to ameloblasts will rescue enamel/root defects in *op/opS* mice. CSF-1 is the growth factor for cells of the monocyte-macrophage lineage.

Role: Co-Investigator

Senlin Li's Research Projects

Stem cells are critical to cellular homeostasis and thus our health. Stem cell exhaustion is a hallmark of aging that is one of the greatest risk factors for the majority of human diseases. My primary research interest is the development of stem cell-based therapies for a variety of hard-to-treat diseases. Our current efforts are to establish a novel platform that enable autologous hematopoietic stem cell transplantation (HSCT)-based cell and gene therapy, including HSC-derived macrophage gene therapy for neurodegenerative diseases such as Parkinson's (to deliver neurotrophic factors to the brain) and atherosclerosis (to provide antiatherogenic and/or atheroprotective factors *per se*). The platform HSCT technology is non-cytotoxic/-cytotoxic and essentially free of risk. This invention, combined with the latest genome editing technologies (CRISPR/Cas9, in particular), will significantly increase the willingness of patients and their physicians to apply HSCT for the treatment of disease. We are expanding our research focus to include inherited and acquired blood/immune disorders such as HIV/AIDS (by genome editing to knockout CCR5-/- and thus HIV-resistant blood cells) and chronic granulomatous disease (by various incorporations of the missing gene in order to achieve expression in phagocytes). With the same platform, we are also working on the amelioration of renal fibrosis and blood/immune rejuvenation. Finally, we are interested in production of unlimited youthful HSCs through generation of personalized iPS cells followed by their differentiation.